## Remarks

Claims 1-9 and 19 are under consideration and have been rejected. In response, applicants amend independent claims 1 and 19, and provide arguments in favor of removing the rejections. Reconsideration and allowance are solicited.

### Drawings

Drawing changes are being made. Applicants plan to submit corrected drawings later this month.

### The Specification

On the top of page 3 of the office action the Examiner has noted that:

I. page one of the specification is not numbered. In response, this page has been numbered.

II. SEQ ID numbers are missing from the specification. In response, SEQ ID numbers have been added to pages 8, 14, and 43 of the specification.

III. The abstract has been objected to. In response, applicants have clarified line 18 of the abstract as requested.

### Rejections Based on Indefiniteness

Several indefiniteness rejections have been asserted on page 4 as follows:

A. The term "complex form" is deemed unclear. In response, this term has been amended to recite "complex formed."

B. A rejection has been made from an argument that the claim does not clearly correlate complex formation to sperm activity. In response the phrase " complex formed from step (a) as a measure of sperm activity" has been added.

C. Claims 1 and 9 are deemed vague from use of the terms "appropriate concentration," and "appropriate amount" In response, these terms have been deleted. Claim 1 is also rejected because of the term "conditions permitting." This term is clarified by addition of further language to the claim.

D. Claim 19 has been rejected on indefiniteness grounds. The Examiner suggested that "actual reagents be included in the claim for clarity." In response, applicants have amended this claim to recite representative reagents. The term "Ni-NTA resin" is supported, for example on page 45, line 32. The terms "washing buffer" and "calcium ionophore control is supported, for example, on pages 45 line 32 and 48 lines 21-24, respectively.

Reconsideration and allowance in view of the amendments earnestly is solicited.

Rejections based on Anticipation and Obviousness

Claims 1-9 stand rejected on alleged anticipation grounds in view of VanDuin. However, the claim element "human zona pellucida protein 3" is not found in this reference.

Taught only 607 page 1st column last line Van Duin

The claim element "recombinant human zona pellucida protein 3" recited in claims 1 and 19 and dependent claims thereon is a glycoprotein, not a protein. The protein part of this molecule has a certain sequence that is specific to the human. The carbohydrate part of this molecule has a certain sequence (branched structure) that is specific to the human. > NOT recited in claims.

This claim term does not mean a human sequence polypeptide with a non-human carbohydrate. This claim term also does not mean a non-human sequence polypeptide with a human oocyte carbohydrate. This term as used in the present claims requires that both parts are human, and, preferably oocyte like. Applicants cannot recite the exact branch structure of their carbohydrate nor do they have to. Applicants are not obligated to know how their invention works but have provided enough information to allow others to practice the invention. The "human" zona pellucida protein described in the cited references is not this molecule. At best the protein part may have the same sequence, but the carbohydrate part differs greatly.

The alleged anticipating reference by VanDuin admits (see abstract) that the carbohydrate differs greatly from the human oocyte form ("has a molecular mass +-5kDa smaller than that of natural ZP3.") In this reference, the "human" glycoprotein is termed "natural." Applicants did not choose to utilize the same terminology but instead emphasize this very same characteristic throughout the specification as the real biological activity. See for example, page 22 line 19-20 "[t]o insure biological activity of ZP3, the human ovarian cell was used to express the recombinant ZP3." Applicants point out that the product of their cell expression is the same as what VanDuin calls "natural." Applicants are unaware of any convincing science or scientific argument that their product is not this so-called "natural" form. natural or distinguishing characteristic not recited recombinant claims require sperm + rec ZP3.

VanDuin studied non-natural material and used hamster oocytes that were missing zona, in order to overcome the poor biological activity of the non-natural material. The specific human egg and sperm interaction, which was the focus of applicants' study was not addressed sufficiently, because the material did not have the "biological activity" referred to in the present specification. Accordingly, the material as claimed differs not only structurally, but also differs greatly in function. Not claimed

All prior art references asserted concern the different glycoprotein studied by VanDuin and which VanDuin admits differ structurally (smaller size presumably due to smaller carbohydrate) from that recited in the claims. Accordingly, prima facie obviousness is not possible here as well, because none of the references, either alone or in combination recite the glycoprotein created by applicants, studied by applicants, and recited in applicants' claims.

Because an element found in all claims is missing from all cited reference, reconsideration and removal of the prior art rejections earnestly is solicited.

**Conclusion**

In view of the foregoing remarks and amendments, reconsideration and allowance of the remaining claims are requested. If any issues remain that could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner respectfully is requested to contact the undersigned.

Respectfully submitted,

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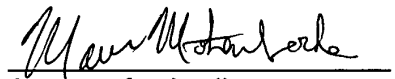

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MARKED UP COPY OF AMENDMENTS



In the Specification:

Page 1, before line 1, insert the following page number 1.

Page 8, second paragraph (lines 11-21, delete the existing paragraph and insert the following new paragraph:

Fig. 2. Determination of expression of recombinant ZP3 in transfected PA-1 cells by RT-PCT. (A RT-PCT amplification of first strand of cDNA from the RNA sample of PA-1 cells stable transfected with human ZP3 CDNA with primers A9CH1)/B(CH2) and A/C (B1). Location of PR primers (Primer A5' - TAGGATCCACCATGGACTGAGCTATAGG-3', SEQ ID NO: 1, primer B5'-TTATTCGGAAGCAGACACAGGGTGGGAGGCAGT-3', SEQ ID NO: 2, Primer C 5'-
TTCTCGAGTTAATGATGATGATGATGTTCGGAAGCAGACACAGGGTGG GAGGCAGT-3') SEQ ID NO: 3

Page 14, second paragraph (lines 13-37), delete the existing paragraph and insert the following new paragraph:

Total RNA was isolated from the human ovary (the utilization of human tissue was approved by the Institutional Review Board of Eastern Virginia Medical School) by using the guandium thiocyanate method (Chirgwin, et al, 1979). A pair of primers was designed based on the published sequence of hZP3 cDNA with additional restriction enzyme sites and histidine tail (Chamberlin and Dean, 1990). The sense primer was located between base 1 to 22 with Bam HI site in the 5' end (5'-TAGGATCCACCATGGAGTGAGCTATAGG-3') SEQ ID NO: 4. The antisense primer was located between base 1256 and 1262 (5'-

TTCTCGACTTAATGATGATGATGATGAGATGTTCGGAAGCACACACAGGGTC GGAGGCAGT-3') SEQ ID NO: 5. A SEQUENCE OF Xho I restriction site and a sequence coding for six histidine residues were introduced into 5' end of this primer for the purpose of the purifying the recombinant protein as well as for subcloning. RT-PCR of the mRNA samples from human ovaries revealed a single band of approximately 1,300 bases. This PCR product was purified and inserted into a mammalian cell expression vector, pcDNA 3.1 (Invitrogen, Carlsbad, CA). The positive clone was sequenced and found to be identical to those of the published hZP3 (Chamberlin and Dean, 1990).

Pages 43 bridging 44, last paragraph (lines 31-24), delete the existing paragraph and insert the following new paragraph:

Isolation of human ovarian mRNA and construction of cDNA for human ZP3-Total RNA was isolated from the human ovary by using the guanidinium thiocyanate method. A pair of primers was designed based on the published sequence of hZP3 cDNA with additional restriction enzyme sites and a histidine tail (12). The sense primer is located between bases 1 to 22 with Bam HI site in the 5' end (5'-TAGGATCCATGGAGCTGAGCTATAGGC-3') SEQ ID NO: 6. The antisense primer is located between base 1256 and 1262 (5'-TTCTCGAGTTAATGATGATGATGATGATGTTCGGAAGCAGACACAGGGTGG GAGGCAGT-3') SEQ ID NO: 7. A sequence of Xho I restriction site and a sequence coding for six histidine residues were introduced into 5' end of this primer for the purpose of the purifying the recombinant protein as well as for subcloning. Reverse transcription-polymerase chain reaction (RT-PCR) of the mRNA samples from the human ovary revealed a single band of approximately 1,278 bp. This PCR product was further characterized by restriction mapping, Southern blotting and sequencing analysis demonstrating identical composition to be published human ZP3(16). The PCR product was inserted into a mammalian cell expression vector, pcDNA 3.1 (Invitrogen, Carlsbad, CA). An in vitro transcription and translation system (Reticulocyte Lysate System; Promega,

Madison, WI) was used to determine the molecular weight of the (non-glycosylated) protein core of the recombinant ZP3.

In the Abstract

Page 68, (lines 11-31) delete the existing Abstract and replace with the following:

The present invention provides a method to determine sperm activity comprising the steps of: (a) contacting an appropriate concentration of human zona pellucida protein 3 with an appropriate amount of sperm under conditions permitting the formation of a complex between the human zona pellucida protein 3 and the sperm; and (b) determining the complex formed. The invention further provides a method to determine sperm activity comprising the steps of (a) contacting an appropriate concentration of human zona pellucida protein 3 with an appropriate amount of sperm under conditions permitting an acrosome reaction to occur; and (b) determining the extent of the acrosome reaction. Finally, this invention provides a diagnosis kit for sperm activity comprising three (3) compartments with (a) an appropriate amount of human zona pellucida protein 3; (b) the reagents used for establishing the conditions for allowing the binding of sperm; and (c) the reagents used for establishing the conditions for allowing an acrosome reaction.

In the Claims:

1. A method to determine human sperm activity with human ova, comprising the steps of:

(a) contacting [an appropriate concentration of] recombinant human zona pellucida protein 3 with an appropriate amount of sperm under conditions permitting the formation of a complex between the human zona pellucida protein 3 and the sperm; and

(b) determining the complex formed from step (a) as a measure of sperm activity.

19. A diagnosis kit for sperm activity comprising compartments with (a) [an appropriate amount of] recombinant human zona pellucida protein 3 and (b) [the] one or more reagents selected from the group consisting of binding buffer, Ni-NTA resin, washing buffer, and a calcium ionophore control [used for establishing the conditions for allowing an acrosome reaction].

IN THE ABSTRACT

The present invention provides a method to determine sperm activity comprising the steps of: (a) contacting an appropriate concentration of human zona pellucida protein 3 with an appropriate amount of sperm under conditions permitting the formation of a complex between the human zona pellucida protein 3 and the sperm; and (b) determining the complex formed. The invention further provides a method to determine sperm activity comprising the steps of (a) contacting an appropriate concentration of human zona pellucida protein 3 with an appropriate amount of sperm under conditions permitting an acrosome reaction to occur; and (b) determining the extent of the acrosome reaction. Finally, this invention provides a diagnosis kit for sperm activity comprising three (3) compartments with (a) an appropriate amount of human zona pellucida protein 3; (b) the reagents used for establishing the conditions for allowing the binding of sperm; and (c) the reagents used for establishing the conditions for allowing an acrosome reaction.